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In vitro evaluation of fungitoxicants and Phyto-extracts against *Neovossia indica* (Mitra) Mund. the causal agent of Karnal bunt of wheat

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ABSTRACT

An effective management practice was developed; thirteen fungitoxicants and six phyto-extracts were evaluated under laboratory condition. *N. indica* was isolated from infected wheat grains. Among the systemic fungicides, Tilt 25 EC at 200 ppm was adjudged best to check mycelia growth and teliospore germination of fungus. In case of non-systemic fungicides, Dithane M-45 was found highly effective at 2000 ppm. Among the phyto-extracts tested in laboratory, *Lantana camara* at 75 per cent concentration was found best for growth and teliospore germination inhibition of *N. indica*.

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INTRODUCTION

The geographical concentration of wheat is found between 30-55ºN latitude in the Northern hemisphere and between 20-40ºS in Southern hemisphere (Anonymous, 2011). Karnal bunt incited by *Neovossia indica* is one of the most important diseases of wheat crop. Karnal bunt was first reported by Mitra in 1931 from Karnal (Haryana). The disease might have been observed by Haward and Haward in 1909 from Layallpur (now Faisalabad, Pakistan) was not properly diagnosed. It is widespread in northwest India and in adjacent areas of Pakistan and Afghanistan (Wiese, 1998). In 1996, the disease was detected in Arizona in certified durum wheat seed. The USDA also detected Karnal bunt in grain shipments from Lebanon and Syria (USDA, 2003). *Neovossia indica was* isolated from infected wheat grains. The efforts were made to evaluate the effect of fungicides on radial growth and teliospore germination against *N. indica*. In this context, thirteen fungicides *viz.*, systemic fungicides (Tilt 25EC, Raxil 2DS, Contaf 25EC, Folicur 25EC, Baycor 25WP, Vitavax 75WP and Bavistin 50WP), contact fungicides (Captan 50WP, Thiram 75DS, Kavach 75WP and Dithane M-45) and co-ordinated fungicides (Moximate 72WP and F-100) were evaluated at different concentrations. Many plants are used as antimicrobial agent. Antimicrobial properties of these plants are due to their alkaloids, steroids, natural phenols, terpenes, sesqueterpens and related compounds. Evaluation

of various phytoextratcs against *N. indica* under laboratory conditions, leaves and rhizomes (powder) of plants namely *Curcuma longa, Ageratum conyzoids, Calotropis procera, Eupatorium adenophorum, Eucalyptus globules* and *Lantana camara* were collected and extracted in sterilized water. The sterilized water extracts of different concentrations (25, 50, 75 and 100%) were tested for inhibitory activity on mycelial growth and teliospore germination of *N. indica.*

MATERIAL AND METHODS

I-Mycelial growth inhibition test :

Thirteen fungicides (systemic, non-systemic and co-ordinated fungitoxicants) were evaluated at different concentrations against *N. indica* by Poisoned Food Technique (Schmitz, 1930). Stock solutions of fungitoxicants were prepared in sterilized distilled water by dissolving double quantity of the fungitoxicants required in measured volume of sterilized distilled water. Calculated volume of stock solution was then added to double concentrated sterilized PDA so as to get the final concentrations of 25, 50, 100, 200 and 250 ppm for systemic fungitoxicants. Similar procedure was followed for non- systemic fungitoxicants for obtaining concentrations of 250, 500, 1000, 2000 and 2500 ppm. The stock solution was added to sterilized distilled water for getting above concentrations. The medium containing different concentrations of fungitoxicants was poured (20ml) in each sterilized Petri plate and allowed to solidify. Each Petri plate was centrally inoculated with 10mm mycelial disc dissected with the help of sterilized cork borer from 15 days old culture of test fungus and unammended plates served as check. Three replications were maintained for each treatment and incubated at 20±1°C. Regular observations were recorded and finally the colony diameter was measured 25 days after inoculation. Per cent inhibition of mycelial growth was calculated by Vincent (1947) formula.

II-Teliospores germination Inhibition test :

Double strength suspension of systemic fungitoxicants (25, 50, 100, 200 and 250ppm) and non-systemic fungitoxicants (250, 500, 1000 and 2000ppm) were separately prepared in sterilized distilled water. Teliospores were taken from infected seed and suspended in sterilized distilled water. Teliospore suspension (0.5ml) was added to 0.5ml of fungicidal suspension (double strength) in a test tube and shaken well. A drop of this mixture was placed in cavity slide and incubated at 20±1°C in humidity chamber created in 100mm plastic Petri plate containing two layers of moist filter paper. Three replications were kept under each treatment. Teliospore germination was counted after 7 days of incubation. Teliospore germination in sterilized distilled water served as control (Reddick and Wallace, 1910). Per cent inhibition of teliospores was calculated as per Vincent (1947) formula :

$$\mathbf{I(\%) = \frac{(C - T)}{C} \times 100}$$

where,

I = Per cent inhibition of teliospores

C = No. of germinated teliospores in control.

T = No. of germinated teliospores in treatment

Six locally available plants were used for testing their efficacy against *N. indica* by preparing water extracts (Table 3). Method follow as per fungitoxicants for mycelium growth and teliospores germination inhibition.

RESULTS AND DISCUSSION

All the systemic fungitoxicants significantly inhibited the radial growth of *N. indica in vitro* over check. However, maximum inhibition (94.41%) of mycelial growth was achieved with Tilt 25EC followed by Raxil 2DS (94.28%) and Folicur 25EC (93.01%) at 200 ppm and rated at par (Table 1). There are limited reports on the evaluation of fungicides against mycelial growth of *N. indica* in *in vitro.* Krishna (1979) reported almost complete inhibition of *N. indica* with Bavistin 50WP at 25 ppm concentration. Singh (1984) reported that Bavistin 50WP and Vitavax 75WP were highly inhibitory to the mycelial growth of *N. indica* at10 ppm concentration. However, in the present study all the test fungicides at high concentrations (250 ppm) resulted in complete inhibition of mycelial growth of pathogen. Tilt, Raxil, Folicur and Contaf at 200 ppm were adjudged best fungicides for inhibitory mycelial growth of *N. indica* (Kapadiya *et al.*, 2013; Balai and Singh, 2013). However, co-ordinated fungicides were found to be least effective.

Among non-systemic fungitoxicants Dithane M-45 gave maximum inhibition (92.11%) of mycelial growth followed by Kavach (89.25%) and Captan (88.48%) at 2000 ppm differed significantly from each other. Non-systemic fungitoxicants Dithane M-45 at 2000 ppm was found most effective in inhibiting mycelial growth of *N. indica*. However, Krishna (1979) reported that Thiram 75DS at 100 ppm was completely inhibitory to the mycelial growth of pathogen. Since there is no other report related to *in vitro* evaluation of fungicides against *N. indica*, hence results could not be compared.

Tilt 25EC also gave cent per cent inhibition of teliospore germination of pathogen followed by Raxil (95.41%), Folicur (97.35%) at 200 ppm concentration. In case of non-systemic, Dithane M-45 resulted in 85.85 per cent inhibition of teliospore germination. However, Kavach and Captan gave 77.30 and 67.28 per cent inhibition, respectively at 1000 ppm concentration (Table 2).

Rai and Singh (1979) reported partial effectiveness of Bavistin 50WP in inhibiting teliospores germination of *N. indica*. However, Singh (1987) reported that Bavistin 50WP and Vitavax 75WP+ Thiram were completely inhibitory to teliospores of *N. indica.* In the present study Bavistin 50WP and Vitavax 75WP at 250 ppm were found completely inhibitory to teliospore germination of *N. indica*. Tilt 25EC, Raxil 2DS and Folicur 25EC at low concentration (200ppm) were adjudged best fungicides for inhibiting teliospore germination of the pathogen. Rivera-Castaneda *et al.* (2001) reported that few of the commercial fungicides were found effective in inhibiting teliospore germination of *N. indica*. Thiram 75DS in combination with Bavistin 50WP and Vitavax 75WP were found highly

Table 1 : Mycelial growth and teliospore germination inhibition of *Neovossia indica* by systemic fungitoxicants incorporated in PDA and incubated at 20±1 ^{0}C

Fungicide	Teliospore germination inhibition (%) at different concentrations (ppm)					Mycelial inhibition (%) at different concentrations (ppm)				
	25	50	100	200	250	25	50	100	200	250
Tilt 25EC	42.04*	60.50	83.42	100	100.00	45.71*	55.36	81.44	94.41	100.00
	(40.28)	(51.14)	(66.41)	(89.96)	(89.96)	(42.52)	(48.05)	(64.43)	(76.32)	(89.96)
Folicur	19.45	37.44	64.41	97.35	100.00	42.91	54.76	80.64	93.01	100.00
25EC	(25.53)	(37.58)	(53.49)	(84.50)	(89.96)	(40.91)	(47.71)	(63.87)	(74.66)	(89.96)
Contaf	14.44	27.02	46.06	67.50	100.00	41.32	54.36	78.37	91.62	100.00
25EC	(22.12)	(31.21)	(42.66)	(55.67)	(89.96)	(39.98)	(47.48)	(62.26)	(73.16)	(89.96)
Baycor	9.08	11.05	33.09	56.89	100.00	40.12	53.76	79.44	89.82	100.00
25WP	(17.44)	(18.85)	(35.08)	(49.01)	(89.96)	(39.28)	(47.13)	(63.01)	(71.38)	(89.96)
Vitavax	12.37	22.43	44.29	63.59	100.00	40.12	54.29	77.57	90.22	100.00
75WP	(19.74)	(27.44)	(41.65)	(53.04)	(89.96)	(39.28)	(47.44)	(61.71)	(71.76)	(89.96)
Bavistin	18.78	36.27	62.03	95.41	100.00	38.92	53.09	77.51	89.62	100.00
50WP	(25.68)	(37.01)	(53.26)	(83.96)	(89.96)	(38.58)	(46.75)	(61.66)	(71.18)	(89.96)
Raxil 2DS	5.28	18.90	38.39	63.34	100.00	44.28	55.22	80.44	94.28	100.00
	(12.89)	(25.88)	(38.15)	(52.73)	(89.96)	(41.70)	(47.98)	(63.72)	(76.14)	(89.96)
Moximate	4.18	14.72	36.49	60.17	100.00	36.00	50.03	77.22	89.13	100.00
2WP**	(11.17)	(21.84)	(36.08)	(51.10)	(89.96)	(37.56)	(45.52)	(61.15)	(71.04)	(89.96)
F-100**	4.78	15.84	36.88	60.29	100.00	36.39	51.46	78.23	89.29	100.00
	(11.83)	(22.34)	(36.85)	(51.79)	(89.96)	(37.73)	(45.90)	(61.69)	(71.37)	(89.96)
Control	-	-	-	-	-	-	-	-	-	-
C.D. (P=0.05)	9.26	7.25	10.59	8.22	1.14	2.19	0.50	0.42	1.77	0.64

*Average of three replications, Figure in parenthesis are arc sine transformed values, ** Co-ordinated fungicides

Table 2 : Mycelial growth and teliospore germination inhibition of *Neovossia indica* by non-systemic fungicides

Fungicide	Teliospore inhibition (%)* at different concentrations (ppm)				Mycelial inhibition (%) at different concentrations (ppm)				
	250	500	1000	2000	250	500	1000	2000	2500
Thiram 75DS	17.32 *	36.11	66.28	100.00	34.54	51.09	75.64	87.48	100.00
	(24.53)	(36.86)	(54.48)	(89.96)	(35.97)	(45.61)	(60.40)	(69.26)	(89.96)
Captan 50WP	6.72	32.79	67.28	100.00	35.26	52.45	76.18	88.48	100.00
	(14.26)	(34.67)	(55.08)	(89.96)	(36.41)	(46.38)	(60.76)	(70.13)	(89.96)
Kavach 75WP	11.36	47.84	77.30	100.00	40.59	53.09	78.27	89.25	100.00
	(19.60)	(43.69)	(61.60)	(89.96)	(39.56)	(46.75)	(62.19)	(70.83)	(89.96)
Dithane M-45	35.14	57.13	85.85	100.00	42.32	54.26	79.64	92.11	100.00
	(36.29)	(49.09)	(68.08)	(89.96)	(40.56)	(47.42)	(63.15)	(73.66)	(89.96)
Control	-	-	-	-	-	-	-	-	-
C.D. (P=0.05)	7.39	11.07	5.49	1.14	0.38	0.49	0.63	0.67	NS

*Average of three replications, Figure in parenthesis are arc sine transformed values, NS = non significance

Table 3 : Teliospore germination and mycelia growth inhibition of *N. indica* by different phyto-extractsat 20±1°C

Phyto-extracts	Teliospore inhibition (%) at different concentrations (%)				Mycelial growth inhibition (%) at different concentrations (%)			
	25	50	75	100	25	50	75	100
Curcuma longa	13. 54* (21. 96)	57.63 (51. 04)	91.82 (73.35)	100 (89.96)	39.09 (38.68)	81.69 (64.66)	91.36 (73.18)	100 (89.96)
Ageratum conyzoids	14.29 (22.20)	60.84 (51.24)	91.70 (73.23)	100 (89.96)	40.12 (39.27)	78.81 (62.59)	91.56 (73.29)	100 (89.96)
Calotropis procera	12.42 (20.63)	63.81 (53.00)	100.00 (89.96)	100 (89.96)	41.15 (39.89)	83.33 (65.93)	91.77 (75.52)	100 (89.96)
Eucalyptus globules	11.87 (20.14)	74.32 (59.54)	100.00 (89.96)	100 (89.96)	46.71 (43.09)	82.72 (65.44)	93.42 (75.75)	100 (89.96)
Eupatorium adenophorum	13.37 (21.44)	65.14 (53.79)	100.00 (89.96)	100 (89.96)	44.03 (41.55)	65.02 (53.75)	91.56 (73.10)	100 (89.96)
Lantana camara	10.35 (18.75)	82.54 (65.28)	100.00 (89.96)	100 (89.96)	47.78 (43.98)	80.04 (63.48)	94.24 (76.10)	100 (89.96)
Control	-	-	-	-	-	-	-	-
CD (P=0.05)	0.65	1.40	0.37	0.64	2.88	3.51	4.91	1.14

*Average of three replications, Figure in parenthesis are arc sine transformed values

effective in inhibiting teliospore germination (Singh, 1987; Chaudhary and Chaudhari, 2013). However, in present investigation, Thiram 75DS alone was found least effective in inhibiting teliospores germination. Dithane M-45 at 1000 ppm was recorded most effective fungicides against teliospores germination of *N. indica*.

All the botanicals evaluated against *N. indica* resulted in significant reduction in radial growth of pathogen over check. However, maximum inhibition was observed in *Lantana camara* (94.24%), *Eucalyptus globulus* (93.42%), and *Calotropis procera* (91.77%). *Eupatorium adenophorum*, *Ageratum conyzoids* and *Curcuma longa* resulted in 91.56, 91.56 and 91.36 per cent inhibition of mycelial growth, respectively at 75 per cent concentration (Table 3). All the botanicals were equally effective as they did not show any significant difference in their efficacy. All the test phytoextracts at 75 per cent concentration were statistically rated at par in growth inhibition of *N. indica*. There is no available report on *in vitro* antifungal activity of phytoextracts against *N. indica*. However, antifungal activity of *L. camara* and *E. adenophorum* against *R. solani* has been reported by Goswami (2008) and Pandit (2010). Nane and Thapliyal (1993) reported that the presence of phenolic compounds in Eucalyptus may be responsible for its antifungal properties.

Aqueous extracts of *L. camara, E. globulus, E. adenophorum* and *C. procera* resulted in complete inhibition of teliospore germination of pathogen at 75 per cent concentration. *C. longa* and *A. conyzoids* gave 91.82 and 90.70 per cent inhibition, respectively at same concentration. The inhibitory effect of *L. camara* on teliospore germination may be attributed due to the presence of antisporulant compound like *Pentacyclic triterpenoids* (Barceloux and Donald, 2008). Antifungal activity of *E. adenophorum* and *L. camara* against plant pathogen has also been reported by earlier workers (Chaudhary and Chaudhari, 2013; Chakrabarty *et al*., 2013 and Bhardwaj and Sahu, 2014).

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R. dominica was reared on sorghum grains (variety GJ-42) from June 9, 2012 to May 26, 2013, during that period total eight generations were completed. time span.

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